COACERVATION OF STARCH

by

ROBERT EARL BAKER

B. S., Kansas State University, 1965

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain
Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1967

Approved by:

Major Professor

TABLE OF CONTENTS

INTRODUCTION

In homogeneous liquid systems, a demixing into two liquid, isotropic layers takes place under certain conditions. In most cases the two layers are quite different in composition (e.g. in the system ether-water) (59).

A phase separation also occurs sometimes in solutions of colloids, for example in solutions of gelatin upon addition of alcohol or salts (6, 11, 12, 13). The two phases in this case differ mainly in the concentration of gelatin.

For this kind of phase separation, Bungenberg de Jong and Kruyt (35) introduced the name coacervation. The more concentrated phase is called the coacervate. The dilute phase is called the equilibrium liquid. The above mentioned phase separation is considered by Bungenberg de Jong to be caused by the dehydrating action of the added alcohol, leading to a reduction in solubility of the gelatin. The presence of charged groups plays little or no part in the phenomenon. On the other hand, if one mixes solutions of oppositely charged polyelectrolytes (e.g. gelatin and gum arabic between pH 1.7 and pH 4.7), coacervation is observed even without addition of alcohol. From these two examples we see that coacervation may be subdivided into two main groups (59): (a) simple coacervation (in which charged groups play no part), (b) complex coacervation (in which opposition of charge is a necessary condition for phase separation). This division is not an ideal one in so far as only one mechanism is placed in the foreground each time. Cases are known in which both are in action at the same time and one could in those cases speak of mixed types. In some

cases one of the mechanisms is well to the fore; for example in the coacervation of isoelectric gelatin with alcohol, one is mainly concerned with simple coacervation, although there are indications that interaction between charges of opposite sign also plays a part to a slight extent (35). In other cases, both mechanisms clearly act simultaneously; for example in the coacervation of gum arabic + electrolyte + alcohol. In this case the alcohol has a two-fold action, on the one hand it reduces the solubility of the non-ionized groups, on the other hand it strengthens the interaction between the cations of the added salt and the negatively ionized groups of the gum arabic. Also the main division has nothing to do with the number of colloidal components present. Cases are known in both main groups in which coacervation occurs when two colloids are present as well as when only one colloid is present.

If one adds a little HCl to a warm mixture of dilute gelatin and gum arabic sols, complex coacervation first takes place at a certain pH value below 4.8 (the isoelectric point of the gelatin). The liquid becomes turbid and the presence of coacervate drops can be readily detected microscopically. If now one adds some NaOH to attain a pH above 4.8, the coacervate disappears to recur again after reacidification. The described reversibility of complex coacervation is not restricted to the combination gelatin-gum arabic but holds generally, provided no secondary changes of a different kind occur. The coacervate is reversible upon change in temperature, disappearing on warming and reappearing again on cooling of the solution. According to Bungenberg de Jong (6) complex coacervation depends on the pH, the mixing

proportions of isohydric sols, the initial concentrations of the isohydric sols, and the possible presence of indifferent salts.

If one introduces a coacervated system, consisting of coacervate drops suspended in their equilibrium liquid, into a direct current electric field three phenomena can be observed simultaneously: electrophoresis, deformation, and disintegration. Electrophoresis is the migration of a charged particle under the influence of an electrical field. Deformation is the flattening of the coacervate drops in a direction perpendicular to the lines of force of the field. In disintegration, the two colloids forming the drop tend to separate leading to a disappearance of the drop.

Several instances of starch coacervation have been demonstrated, though there is an adverse suggestion that starch fails to form a coacervate (32). This is attributed to the weak charge which starch molecules generally show when dispersed in water. However, the use of freezing or the addition of ethanol or chloral hydrate to bring about coacervation of starch pastes present interesting methods of preparing starch coacervates (3, 18, 39, 63). Starch sponge produced by freezing of starch paste has been found to be a useful medical material (3, 56). It has been suggested that the failure of starch used as a thickening agent in soups and gravies is due to the formation of starch coacervates. There has also been a suggestion that the loss of sizing power of starch pastes is due to coacervation. The theory has been presented that starch granule formation takes place by means of coacervation of the carbohydrate within the plant cell (39). The coacervate formed is then considered to undergo rhythmic crystallization to form

the starch granule.

Starch coacervation obtained by freezing or by addition of ethanol, salts or chloral hydrate has been studied in this department (18, 63). This thesis presents the results of further work on starch coacervation. The aim of this work was to provide more information on the conditions leading to the formation of starch coacervates and on the properties of these coacervates. The results of electrophoretic studies of the starch coacervate drops and the possible implications of these results are presented in this thesis. Work on the effect of neutral salts in the formation and stability of the coacervate drops is also presented.

REVIEW OF LITERATURE

In the older literature, cases of partial miscibility, in particular in solutions of protamines, have been described without causing any surprise (34). Other cases of separation into two liquid layers were gradually discovered, but they began to attract the attention of investigators only when the opinion became more and more general that the behavior of all colloids depended upon boundary phenomena. According to this view, a sol always had to be considered as a two-phase system in which one phase (the disperse phase) is very finely divided in the bosom of the second (the dispersion medium or continuous phase). The striking fact that sols belong more or less clearly to two different types led Wo. Ostwald to assume that the outstanding differences between these two types of sols were based on the solid and the liquid nature respectively of the dispersed phase. Ostwald based on this his classification of the sols into "suspensoids" and "emulsoids" and he saw in the partial miscibility phenomena which occur in the latter a direct proof of his conception.

The confusion resulting from the use of a number of different terms for the same phenomenon led Bungenberg de Jong and Kruyt to introduce the term coacervation for this phenomenon. This was taken from the Latin acervus, meaning aggregation or heap and the prefix co, meaning together, to signify the preceding union of the colloidal particles. The more concentrated phase is called the coacervate. Coacervates are colloid-rich liquids which are not spontaneously birefringent. The more dilute phase is called the equilibrium liquid.

In 1938, Irving Langmuir postulated that the formation of tactoids from thixotropic sols, the formation of Schiller layers from iron-oxide sols, the separation of tobacco virus solutions and bentonite sols into two liquid layers, and the crystallization of proteins are all examples of unipolar coacervation which must involve attractive forces. The micelles in unipolar coacervates are not in contact, but are separated by relatively large distances. Either a specific repulsive force or a decrease in Coulomb attraction as the concentration increases, due to decreased charges on micelles, can account for stable coacervates. The general mathematical theory of coacervation presents great difficulties because the approximations of the Debye-Hückel theory cannot be used. In bipolar coacervates, which contain micelles of unlike polarities, the electrical fields and the charges on the micelles increase as the micellar concentration increases. When a certain concentration is reached, the field rises to a value so high as to cause increased hydration which holds the micelles apart and gives stability to the coacervate.

It was shown by Basu and Bhattacharya that explanation of the separation of coacervates followed from the idea of flexibility of chain molecules and the solvent immobilization inside coiled up molecules (2). They considered as a corollary of this viewpoint, that if a molecule were to separate out of the solution in its extended configuration it would give a solid product, whereas if it separated in the coiled-up state, coacervation would take place. This was shown to be true for gelatin or gum arabic precipitated with alcohol.

The influence of various neutral salts on composition and morphology

of coacervates of gelatin and gum arabic has been studied by Bungenberg de Jong and coworkers (6, 7, 11, 13). At a constant pH and constant mixing proportion of the colloids in the total system, the addition of salts causes a change not only of the percentage of water in a complex coacervate, but also in the proportion of colloid in it. The continuous valence rule is applicable to the change of the colloid proportion in which a monovalent cation did not modify the proportion of gelatin to gum arabic in the coacervate, divalent and trivalent cations increased the gum arabic percentage of the coacervate; whereas, divalent and trivalent anions increased the gelatin percentage of the coacervate. The proportion of the two colloids in the equilibrium liquid is modified in a reverse sense from that in the coacervate. A microscopic method was developed for the measurement of changes in diameter of gelatinized coacervate drops. The gelatinized coacervate drops were prepared from a coacervate system containing excess gelatin which upon cooling gave coacervate drops of gum arabic and gelatin contained in a lattice of gelatin. Upon variation of the pH, the diameter of the droplet passed through a minimum which occurred very close to pH 3.7 at which, with the given ratio of mixing of gelatin and gum arabic, complex coacervation is optimum. In regions of low salt concentrations, the changes in diameter are reversible, at higher concentrations irreversible. The reversible change due to the addition of a salt is a swelling. For this swelling, the double valence rule holds. The position of the minimum of the swelling-pH curve, the swelling with salts and the resulting prevalence of the double valence rule are in agreement with the conception that in the gelatinization of the

complex coacervate the typical complex relations have been retained.

It was shown by Voorn and coworkers that theoretically phase separation may arise in solutions of polyelectrolytes due to electrostatic interactions alone (47). The free energy was represented as the sum of an entropy-of-mixing term and an electrostatic-free-energy term, while Van der Waals attractions were neglected. It was shown that the decrease in the electrical free energy in a phase separation can, at given temperature and pressure, more than compensate for the increase in the entropy-of-mixing term. The effects of polymer concentration, dielectric constant, charge density, and salt content follow directly from the thermodynamic treatment of the system. Voorn had shown in an earlier publication that the phase rule was applicable to complex coacervation involving two polyelectrolytes of opposite charge (59). A mathematical model was proposed by Voorn for calculations of complex coacervation based on the interaction of oppositely charged molecular coils in a salt solution.

Coacervates prepared from high polymers of nonelectrolytes have been studied by Dobry (20, 21). Coacervation in this case is due to partial solubility in the solvent used. Dobry has used coacervation as a method of fractionation of high polymers. This may be done in either of two ways, variation of the temperature with constant composition of the solvent, or variation of the composition of the solvent at constant temperature. By using the method of coacervation, fractions of polymers have been prepared which are homogeneous with regard to molecular weight. It is pointed out that this method is much superior to fractional precipitation for preparation of homogeneous solutions of

these polymers. Gavoret and Duclaux have used the method of constant temperature and variation of the composition of the solvent to prepare samples of high polymers of homogeneous molecular weight (27, 28). In this method, the heavy molecules are concentrated in the coacervate while the lighter molecules are concentrated in the excess solvent. Gavoret and Duclaux found that coacervates of a wide variety of high polymers could be prepared by selecting the proper solvent and conditions. Thus this method of fractionation can be applied to a number of polymers which were formerly difficult to prepare with homogeneous molecular weight.

Bamford and Tompa presented the results of their work on the conditions leading to coacervates of nonelectrolytes (1). They considered the thermodynamic aspects of coacervation under these conditions to be completely explained by the current statistical theories of polymer solutions. The system chloroform-alcohol-cellulose was discussed in detail and the theoretical conclusions compared with experimental results. Coacervation was discussed briefly from a molecular point of view by means of phase diagrams.

An extensive study of the behavior of coacervate drops when placed in an electrical field has been made by Bungenberg de Jong and others (10, 16, 17, 35). In alternating fields, the Büchner effect was observed. Circular drops became elliptical in form with the short axis parallel to the direction of the field. This also occurred in direct-current fields to a certain extent. In direct-current fields large vacuoles formed on the trailing side of the drops at some distance under the surface. On the other side of the drops at the extreme edge

a single layer of very small and very closely packed vacuoles was formed and as soon as formed moved in the direction of the poles of the drops. In the mean time, on the side where the coarse vacuolation was found, in the equilibrium liquid outside the drop and at a certain distance from its surface, a number of very small coacervate droplets were formed. The formation of large vacuoles had gone on in their original area and soon the drop was quite filled up with vacuoles. The vacuoles increased in size and number and continually broke through to the outside. The regular form of the drop was totally destroyed. The authors made these observations using special apparatus developed for the purpose (10). The experiments indicated that in the electrical field gum arabic was accumulated at the anodal and gelatin at the cathodal side of the drop as a consequence of electrophoresis of these components within the drop. Thus a separation of the two components of the coacervate occurred within the drop. It appeared from the results of these experiments that this separation rather than changes in pH within the drop was responsible for the variations in interfacial tension reported in an earlier publication (17). Thus the local changes of the interfacial tension between coacervate and equilibrium liquid were not directly responsible for the observed phenomena but only a result of the separation by electrophoresis of the two components within the drop.

Gilbert derived general equations which were used to construct Schlieren patterns for a model system in order to illustrate the types of patterns to which aggregation can give rise. These were given for the electrophoresis and sedimentation of a single substance aggregating reversibly. Although it is not possible for true resolution to occur at the boundary of a system in which equilibrium is maintained, the

shapes of the Schlieren patterns of the diffuse boundaries can be such as to give the impression that partial resolution is occurring.

The results of a series of studies conducted over a period of years were published by Bungenberg de Jong in 1936 (5). In this publication, he showed the similarities between a model made up of coacervates and the living cell. The cell is composed of a number of colloidal systems separated by limiting surfaces that divide it into compartments; the contents of the latter can be, in the extreme case, completely liquid. These compartments are separated by membranes, sometimes quite firm and visible, sometimes liquid and either invisible or difficultly visible. Thus two adjacent compartments, rich in colloids, might be two coacervates, two sols, or even a coacervate and a sol. In the first case, two coacervates different in nature and immiscible might be coexistant; if they were of the same nature, or of a different nature and miscible, the intervention of a third condensed micellar system would be necessary. The case of two sols is identical with that of two miscible coacervates. The coexistence of a sol and a coacervate does not differ in principle from the first case. The simplest combination would be the coexistence of two nonmiscible coacervates or of a coacervate and its equilibrium liquid, for these cases would not require the intervention of a supplementary micellar system to keep them separated.

As early as 1904, Maquenne et al recognized that starch consists of two components which they called amylocellulose and amylopectin (43, 44). Amylocellulose is now called amylopectin and what the authors called amylopectin is now called amylose. Maquenne et al were able to roughly fractionate starch into the two components and found that they

differed widely in susceptibility to enzymes and in their physical properties. These studies were made on potato starch. Roux extended the work to starch extracted from other sources (57). He found that all of the natural starches he examined were composed largely of amylopectin, like common potato starch, and that they contain the amylopectin in about the same percentage. The property which starches possess of forming pastes with boiling water shows that they also contain amylose. Roux concluded that all observations previously made on potato starch are applicable to other native starches.

In 1908, Fouard succeeded in preparing a true solution of starch by filtering a pseudo-solution of partially demineralized starch through a collodion membrane (24, 25, 26). This solution could be defined only by its physical characteristics. The clear, filtered liquid representing 55 percent of the total starch contained in the colloid, gave the typical blue color with iodine. After filtration, the solution showed none of the usual colloidal properties shown by the original pseudo-solution of starch. Fouard concluded that starch is a unique kind of chemical, capable of a complete and reversible physical transformation toward a state of perfect solution.

In studies of gelatinization of wheat starch, Sutra found that the starch could be gelatinized in the cold by chloral hydrate (58). He obtained the same results using chloral hydrate as he did using wheat starch which had been ground for three days in a ball mill.

Dedek et al found that during gelatinization of starch, the electrical resistance increases to a maximum (19). The resistance was measured in a thin layer of starch paste which was being heated at a

constant rate. Dedek considered that as gelatinization proceeded the increase in resistance was due to the binding of free ions in solution by the starch granules.

Wolff and Fernback found an enzyme in the grains of green cereals, which had the property of precipitating soluble starch from its solutions (60, 61, 62). They considered this precipitation to have all the characteristics of a diastatic coagulation and proposed the name amylocoagulase for this diastase. This coagulase was considered to be an essential part of the mechanism by which starch is deposited in a solid state in vegetable cells.

The preparation of condensation products of polyhydroxy compounds with chloral hydrate has been described (4). These were produced by heating the polyhydroxy compound in the presence of chloral hydrate and sulfuric acid. The products were characterized by high viscosity and great stability to heat and acid.

Coacervates formed from glycogen sols under the influence of high concentrations of alcohol have been described by Bungenberg de Jong and coworkers (14). They found that in a narrow range of concentration of alcohol coacervation occurred slowly. In slightly higher concentrations, however, strongly opalescent mixtures were formed, and in very high alcohol concentrations, the glycogen was flocculated. It was considered that the opalescent mixtures could be characterized by the term "coacervate sols". They behaved as hydrophobic sols carrying a small negative charge. Their kinetic units, however, consisted of submicroscopic solvent-poor coacervate droplets. The behavior of the glycogen sols was explained by showing that as the charge per gram of colloid becomes

larger, the chance of an unequal distribution of charged spots on the surface of the particle decreases. The tendency in suitable alcohol concentrations to spontaneous coacervation and formation of coacervate sols also decreases. A study was also made of oriented coacervates made from sols of sizing starch produced by the Huron Milling Company, that separate out as very thin, hexagonal plates to a platelet sol upon heating (8). These were prepared from the sols of sizing starch by addition of high concentrations of alcohol. The formation of other oriented coacervates with other desolvating agents in preparations of amylum soluble was briefly described. The principle of oriented coacervation was considered to be of importance for the crystallization of proteins from their sols.

The separation of immiscible layers from mixtures of gelatin and various starches with or without added salts over a considerably wide range of pH was reported by Ostwald and Hertel (49, 50). These layers must be considered to be coacervates since many of the characteristics of those described correspond with known properties of coacervates. It was found that the volume depended upon the pH and the concentration of added salts if any were present. The maximum volume was found to be at a pH of approximately 3 which would be in the pH range found to be optimum for coacervation.

Koets studied in detail the formation of complex coacervates of amylophosphoric acid and proteins (32, 33). Pure amylose in water is negatively charged, as has been shown by measurements of cataphoresis. The charge is probably due, at least in part, to ionization of hydroxyl groups of the constituent glucose molecules in the surface of the micelle. The charge, however, is small in comparison with that of

other lyophilic colloids, such as agar or gum arabic, in which it is influenced by more strongly dissociated groups resulting from sulfuric acid esters of the carboxyl group. It is probably due to the extreme smallness of its negative charge that unaltered amylose in solution does not show the phenomenon of coacervation with positively charged proteins, the mutual attraction being too small to overcome the repelling force of the micelle hydration. The character of the amylose can, however, be completely changed in this respect by the introduction of strongly negative groups, for instance by esterification with phosphoric acid. The resulting amylophosphoric acid shows an appreciable anodic migration and is able to combine in solution with proteins at such a hydrogen ion concentration that the latter carry a distinct positive charge. The optimum of the attraction of the opposite charges of the micelles is found at a pH of about 3.5.

The formation of coacervates of starch with ethanol, chloral hydrate, and by freezing starch pastes has been reported by MacMasters and others (39). The coacervate formed with chloral hydrate appeared as drops which were tough and elastic. The coacervate was unstable, and within an hour it slowly dispersed into the liquid. After freezing and thawing, all of the pastes, sols, and gels studied showed evidence of having undergone coacervation. In systems containing sufficient starch for gel formation, the coacervate occurred chiefly in sheets and branched forms united to form a continuous spongy mass filling the volume of the original gel. The starch sponge formed in this manner was proposed for use as a surgical agent (3). It was later found to have hemostatic properties and is now on the market as a hemostatic agent (56). Coacervates formed by starch with ethanol or chloral

hydrate have many similarities to the amylose complex formed with butanol.

Rendleman found that the interaction of salts of univalent alkali metals with carbohydrates in anhydrous alcoholic media was rapidly reversible; isolable adducts, probably chelates, were produced whose combining ratios often varied with salt concentration (54, 55). Combining ratio is a function of cation radius, hydroxide concentration, and carbohydrate geometry.

The existance of coacervates of catechol in vacuoles of cells of zinc-deficient plants was reported by Reed and Dufrenoy (51, 52, 53). These spherical inclusions in the cell vacuoles of zinc-deficient plants were formed by a process in which some of the colloidal material in the vacuolar solution of the cell became condensed into a spherical mass instead of being distributed at random in the solvent. Coacervates were found in the vacuoles of cells of roots grown in solutions lacking one or more of the important supplementary micro-elements. Similar bodies, however, were not found in the cells of roots receiving the necessary micro-elements.

Recently, Evreinova, Shurygina, and Oparin have succeeded in carrying out synthesis of starch in coacervate drops of gum arabic and histone (22). This was done by using phosphorylase, glucose-1-phosphate, and a small amount of starch for a primer. It was found that the enzyme was bound to the coacervate drops and that the rate of starch synthesis was 22 to 24 times greater in the drops than in the equilibrium liquid.

Yu and MacMasters reported on the coacervation of corn starch

induced by freezing and by addition of chloral hydrate or one of various salts (63). They found that coacervation occurred in a narrow range between pH 6 and 7. They also found that coacervates could be produced by concentration of the aqueous sol by evaporation of water. To form the coacervates, a 1-percent paste of corn starch was first prepared, then the chloral hydrate or salt was added to the paste. This work was continued by Chung using wheat starch (18). He was able to prepare a coacervate by diluting a 4-percent starch-chloral hydrate solution 1:1 with a 3-percent gelatin solution. The resulting coacervate remained stable on standing for a long period of time at room temperature.

MATERIALS AND METHODS

Materials

A. Starch - About 10 pounds each of three Hard Red Winter wheat flours each from a different variety (Triumph, C.I. 13285, C.I. 13523; Lot Numbers: 65-338, 65-339, 65-340, respectively) were obtained from milling in the pilot mill of the Department of Grain Science and Industry, Kansas State University. One-kilogram lots of each of these flours were used as starch sources. Wheat starch was separated from the flour by the gluten washing method. The flour was made into a stiff dough with distilled water and allowed to stand for 1 hour at room temperature for the gluten to "develop". A small amount of distilled water was added and the dough ball kneaded to wash out the starch. The suspension of starch in water was then poured off and this procedure was repeated until no more starch could be washed out. The impure starch slurry that passed through a No. 11XX nylon sieve was centrifuged at 580 x g. The "tailings" fraction appearing as a layer above the starch was carefully eliminated by scraping and the starch was collected, resuspended in distilled water, recentrifuged and again separated from any trace of tailings. After three such separations, the prime starch was air dried at room temperature for 24 hours. Clumps of starch were broken by hand in order to prevent them from forming a hard mass. The three starches each of which contained 12.5% moisture, 0.30% protein, were used for all experiments.

B. Chloral Hydrate - $C_2H_3Cl_3O_2$, U.S.P. grade, obtained from Fisher Scientific Company was used.

C. Gelatin - A granular gelatin, for bacteriological use, obtained from Fisher Scientific Company was used.

D. Furfural - $C_5H_4O_2$, U.S.P. grade, obtained from Fisher Scientific Company was used.

Methods

A. Coacervation of starch by the action of chloral hydrate and salts - Starch-chloral hydrate solutions were prepared by placing 0.4 gram of starch and 10 ml. of 6M chloral hydrate in a test tube and heating for 45 minutes in a boiling water bath. The solutions were allowed to cool to room temperature before being used. Microscopic examination was made to confirm that complete gelatinization was reached. The resulting solutions were viscous, transparent liquids which upon prolonged standing (more than one month) at room temperature did not acquire any cloudiness or appearance of suspension.

The dilution of each of these liquids with distilled water at pH 3.5 was made in a ratio of 1:8. The water was adjusted to pH 3.5 with 1N HCl. The dilution was carried out at room temperature. Various amounts of 1% solutions of KCl, $CaCl_2$, $Fe_2(SO_4)_3$, and Na_2CO_3, respectively, were added to the distilled water before the addition of the starch-chloral hydrate solution, in an effort to stabilize the unstable coacervate formed.

B. Coacervation of starch by the action of chloral hydrate and furfural - Starch-chloral hydrate solutions were prepared as has been described previously.

The dilution of each of the solutions with distilled water was

made as described above. Various concentrations of furfural were added to the distilled water before the addition of the starch-chloral hydrate solution. The length of time the coacervate remained visible was then measured by microscopic observation.

C. Electrophoresis of starch-chloral hydrate-furfural coacervates - The coacervates were prepared by diluting the starch-chloral hydrate solution in a ratio of 1:8 with distilled water, as above, to which 15% furfural had been added.

A direct-current power supply with voltage range of 0-500 volts and current range of 0-150 milliamperes was used for the electrophoresis. The electrodes were of a paddlewheel design and were made of platinum. Two types of electrophoresis were run, one was free-solution electrophoresis in a U-tube, the other was done in a modification of Bungenberg de Jong's apparatus which can be used to observe electrophoresis under the microscope. Both types of experiment were run at various voltages and current settings and the results observed. The U-tube was loaded by adding 50 ml. of the solution containing the coacervate. The voltage was then set at the desired value and the electrophoresis allowed to proceed. When the modified apparatus of Bungenberg de Jong was used, a few drops of the liquid containing the coacervate were placed on a microscope slide and the apparatus lowered into position. After the experimenter had focused the microscope on the drops, the power was turned on and the results observed through the microscope. For details of construction see Figs. 1 and 2.

D. Electrophoresis of starch-chloral hydrate-gelatin coacervates - The coacervates were prepared by diluting the starch-chloral hydrate

solution in a ratio of 1:1 with a 3% gelatin solution. The gelatin solution was first adjusted to pH 3.5 with 1N HCl. The dilution was carried out at room temperature.

The same two methods of electrophoresis as were described in the previous section were used for study of this coacervate. Various voltage settings were used with both types.

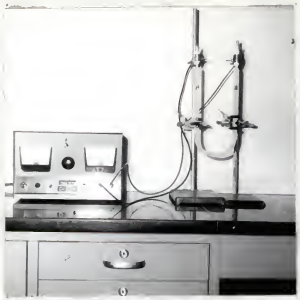

Fig. 1. U-tube and power supply used for electrophoresis of starch coacervates showing details of construction.

Fig. 2. Modified apparatus of Bungenberg de Jong and power supply used in electrophoresis of starch coacervates showing details of construction.

RESULTS AND DISCUSSION

Coacervation of Starch by the Action of Chloral Hydrate and Salts

When four-percent (wt./vol.) of wheat starch was mixed with 10 ml. of 6M chloral hydrate in a test tube and heated in a boiling water bath a viscous transparent liquid was obtained within 45 minutes. The resulting liquid upon prolonged standing (more than one month) at room temperature did not acquire any cloudiness or appearance of suspension. This was confirmed by microscopic examination.

When a four-percent solution (wt./vol.) of wheat starch in chloral hydrate was diluted with distilled water in a ratio of 1:8 at pH 3.5, a white cloudiness immediately occurred but rapidly disappeared into solution within 2 minutes. Microscopic observation indicated that the cloudiness was due to the formation of a large amount of coacervate drops which were nearly transparent and very small in size.

After this period, upon prolonged standing at room temperature, a firm compact white precipitate appeared and gradually increased in quantity during the first 24 hours. The precipitate then settled down onto the bottom of the container, thus two layers distinctly appeared. The white precipitate which has been described by other workers appeared under the microscope as transparent bilobate particles, sometimes with irregularly shaped outlines (18, 39, 63). The precipitate was weakly birefringent and stained blue with KI-I_2 solution whereas the supernatant liquid stained light violet. The weak birefringence when the precipitate was viewed on the polarized light microscope indicated a weakly crystalline structure. Thus this precipitate could

not be considered purely a coacervate. It has been suggested that the precipitate is an amylose-chloral hydrate complex with a large amount of occluded water in its loose texture (18). MacMasters and coworkers indicated the evidence of similarity on the basis of an x-ray pattern of amylose-nitroparaffin complex and amylose-chloral hydrate complex which they obtained as a precipitate of disc- and dumbbell-shaped particles about 10 to 20 microns in diameter or length (39). They further indicated that the complex contained about 23 to 30% chlorine, which decreased to 17% after 9 days. Removal of chloral hydrate from the complex by alcohol extraction resulted in formation of a colorless, friable solid which, after drying, sorbed 159 mg. iodine per gram dry wt. If it is assumed that pure amylose sorbs 200 mg. of iodine g. dry wt., the amylose content of the complex may be considered to be about 80%. This indicates that chloral hydrate may be a good fractionating agent for amylose.

In an effort to stabilize the extremely unstable coacervate formed at first, various concentrations of 1% solutions of one of the salts: KCl, $CaCl_2$, $Fe_2(SO_4)_3$, and Na_2SO_4 were added to the distilled water before the starch-chloral hydrate solutions were diluted (Table 1). The effect of these salts on the stability of the resulting coacervates is shown in Figure 3.

The continuous valance rule described by Bungenberg de Jong in his work with addition of neutral salts to gelatin-gum arabic coacervates was applicable to this coacervate (11, 15). The addition of low concentration of any of the salts seemed to stabilize the coacervate somewhat; but higher salt concentrations made the coacervate more

Table 1. Stability of starch-chloral hydrate coacervates and the quantity of added salt.

KCl	Stability	$CaCl_2$	Stability	$Fe_2(SO_4)_3$	Stability	Na_2CO_3	Stability
Meq.	Min.	Meq.	Min.	Meq.	Min.	Meq.	Min.
0.00	2.00	0.00	2.00	0.00	2.00	0.00	2.00
0.02	6.00	0.03	5.50	0.02	6.00	0.01	6.00
0.05	6.75	0.05	6.50	0.03	6.25	0.02	7.50
0.07	7.00	0.06	6.75	0.04	5.00	0.04	6.00
0.09	7.25	0.07	6.50	0.08	4.50	0.05	5.00
0.11	7.50	0.08	6.25	0.10	4.25	0.08	4.00
0.15	7.75	0.10	6.00	0.15	4.00	0.10	3.00
0.27	8.00	0.27	5.00	0.20	3.75	0.15	2.50
0.35	7.25	0.36	4.75	0.30	3.50	0.20	1.75
0.40	6.50	0.45	4.50	0.40	3.25	0.30	1.50
0.54	6.00	0.54	4.25	0.60	2.25	0.40	1.25
0.67	5.75	0.63	4.00	0.70	1.75	0.60	1.00

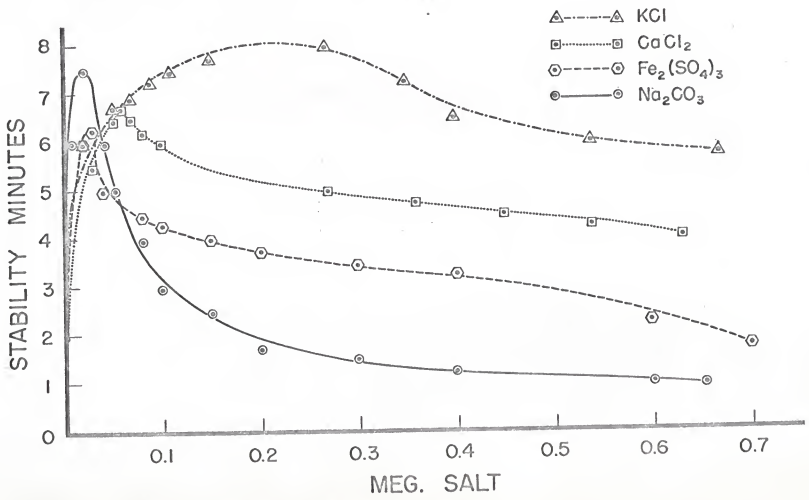

Fig. 3. Effect of salts on coacervated systems.

unstable than it was when no salt was added (Fig. 3). This finding was in accord with Bungenberg de Jong's findings in his work with other coacervates (11). The monovalent cations increased the stability of the coacervate more, although a higher concentration was required, than did the divalent or trivalent cations. This would indicate that the instability of the coacervate was due to an excess negative charge and the effect of the added cations was to neutralize this excess. The decrease in stability at higher salt concentrations was due to over neutralization of the negative charge on the coacervate. Thus the negative charge of the starch-chloral hydrate system was too low to undergo coacervation.

Koets stated that the failure of amylose to form coacervates with positively charged proteins was due to the extreme smallness of its negative charge (32, 33). The mutual attraction was too small to overcome the repelling force of the micelle hydration. In this case the charge was too low for the coacervate to remain stable. Irving Langmuir has shown theoretically that a colloidal ion must have a certain minimum charge for coacervation to occur or, once formed, to remain stable (36).

The increase in stability when a low concentration of Na_2CO_3 was added was due to the monovalent sodium ion (Fig. 3). At only slightly higher concentrations the influence of the divalent carbonate ion was great enough to more than nullify the contribution of the sodium ion to the stability of the coacervate. Thus as the concentration of sodium carbonate was increased there was a rapid decrease in the stability of the coacervate.

The formation of the white precipitate described previously was not influenced by the presence of salt. Approximately the same amount of the precipitate was formed at all salt concentrations. The precipitate when viewed under the microscope was found to be composed of individual round and bilobate particles with irregular outlines. This would be another reason for not considering this precipitate as purely a coacervate, since all known coacervates are sensitive to the addition of neutral salts.

No differences due to variety were found in the ability of these starches to form coacervates. This experiment was carried out using each starch from each variety of wheat individually with each salt.

The aim of this work was to prepare starch coacervates of sufficient stability to be used in work with electrophoresis. Since the maximum length of time the coacervate remained stable was too short to be used in any type of electrophoresis, a different approach was tried to increase the stability.

Coacervation of Starch by the Action of Chloral Hydrate and Furfural

In an effort to stabilize the unstable coacervate formed when the 4% starch-chloral hydrate solution (wt./vol.) was diluted with distilled water at pH 3.5, various concentrations of furfural were added to the distilled water before the starch-chloral hydrate solution was diluted.

When the 4% starch-chloral hydrate solution (wt./vol.) was diluted in a ratio of 1:8 with distilled water to which furfural had been added, a white cloudiness immediately occurred and remained for a length of time depending upon the concentration of furfural added. Under

microscopic examination the initial cloudiness was found to be due to the production of a large number of coacervate drops (Fig. 4). The small drops formed at first came together to form larger drops upon standing for a short time. The appearance of the coacervate was that of a spherical drop with few or no vacuoles. The vacuoles, if present, were usually present singly in the center of each drop. When 15% furfural was added before dilution of the starch-chloral hydrate solution, the resulting coacervate, upon prolonged standing at room temperature, gradually separated to form two distinct layers, one containing practically all of the coacervate drops and the other consisting of the equilibrium liquid.

The higher the concentration of furfural used the longer the drops were stable, up to a point. When 15% furfural was added, the drops remained stable for 24 hours or more. As the furfural concentration was increased above 15% the length of time the drops remained stable decreased.

After the coacervate had redissolved into the equilibrium liquid, the system was allowed to stand at room temperature. A white precipitate began to form and increased in quantity during 24 hours. When observed under the microscope, this precipitate proved to have all the characteristics observed in the precipitate reported previously, which was obtained when a 4% starch-chloral hydrate solution (wt./vol.) was diluted with distilled water and allowed to stand at room temperature. The concentration of furfural used seemed to have no effect on the formation of the precipitate. Approximately the same amount was formed at all concentrations of furfural.

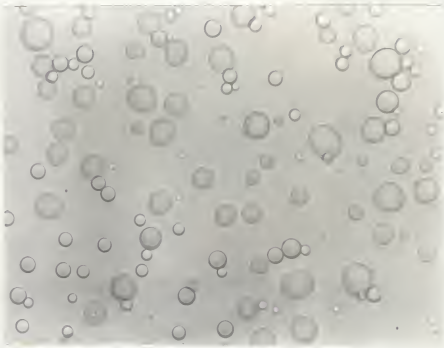

Fig. 4. Starch-chloral hydrate-furfural coacervate drops obtained upon dilution of a 4% starch-chloral hydrate solution 1:8 at pH 3.5 with distilled water to which 15% furfural had been added before dilution. Mag. 300X

The role of the furfural in increasing the stability of the coacervate was to reduce the negative charge of the starch-chloral hydrate system so that coacervation could take place. Thus the concentration of furfural (15%) which gave a coacervate of highest stability of those studied would be the amount of furfural needed to reduce the negative charge to a value which was optimal for coacervation.

The furfural molecule was represented by Finar as being a hybrid of five resonance structures (23). Four of these resonance structures are polar. Thus the positive end of the furfural molecule was available to bring about micelle dehydration by ionic attraction of the two unlike charges. Michaeli and coworkers have shown theoretically that phase separation may arise in solutions of polyelectrolytes due to electrostatic interactions alone (47). Michaeli considered that the free energy of the system was represented as the sum of an entropy-of-mixing term and an electrostatic-free-energy term, while the Van der Waals attractions were neglected. It was shown that for coacervation to take place the decrease in the electrostatic-free-energy term, at a given temperature and pressure, must more than compensate for the increase in the energy-of-mixing term. Otherwise the coacervate formed, if it forms at all, will be extremely unstable. Thus the furfural was able to bring about a large enough decrease in the electrostatic-free-energy term to partially compensate for the increase in the entropy-of-mixing term, and thereby increase the stability of the coacervate.

According to Bungenberg de Jong's classification this coacervate was considered to be a complex coacervate which involved the interaction of a microcation and a colloid anion (35).

Since the coacervate formed upon addition of 15% furfural was stable for 24 hours or more it was suitable for further studies with electrophoresis.

Electrophoresis of Starch-Chloral Hydrate-Furfural Coacervates

Starch-chloral hydrate-furfural coacervates were prepared by dilution of the starch-chloral hydrate solution with distilled water at pH 3.5 to which 15% furfural had been added before the dilution was carried out. The starch-chloral hydrate solution was diluted in a ratio of 1:8.

Two types of electrophoresis were applied to this coacervate. One type was free-solution electrophoresis in which a U-tube was used, and the other used a modification of Bungenberg de Jong's apparatus, which permitted observation of the electrophoresis of the coacervate drop under the microscope.

The results obtained from electrophoresis of the starch-chloral hydrate-furfural coacervate in which the U-tube was used are shown in Figs. 5 and 6. When the solution containing the coacervate was placed in the U-tube, the drops were initially evenly dispersed throughout the solution (Fig. 5). After the electrophoresis had proceeded for a period of two hours or more, depending on the current and voltage setting (Table 2), the coacervate drops had formed a distinct layer near the positive electrode (Fig. 6). This would indicate that the coacervate drops carried a negative charge.

The same direction of movement of the drops was observed when the modified apparatus of Bungenberg de Jong was used. The main

Table 2. Voltage setting and direction of movement of starch-chloral hydrate-furfural coacervates.

Trial No.	Voltage Setting	Direction of Movement			
		After 1 hr.	After 2 hr.	After 3 hr.	After 4 hr.
1	200	?	?	+	+
2	250	?	?	+	+
3	300	?	+	+	+
4	350	?	+	+	+
5	400	+	+	+	+
6	450	+	+	+	+
7	500	+	+	+	+

Table 3. Voltage setting and direction of movement of starch-chloral hydrate-gelatin coacervates.

Trial No.	Voltage Setting	Direction of Movement			
		After 1 hr.	After 2 hr.	After 3 hr.	After 4 hr.
1	200	?	?	-	-
2	250	?	?	-	-
3	300	?	-	-	-
4	350	?	-	-	-
5	400	-	-	-	-
6	450	-	-	-	-
7	500	-	-	-	-

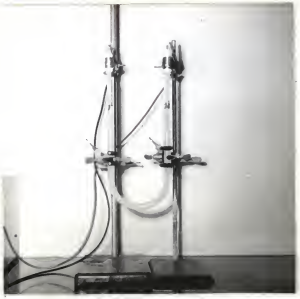

Fig. 5. Appearance of the starch-chloral hydrate-furfural coacervated system when placed in the U-tube prior to electrophoresis.

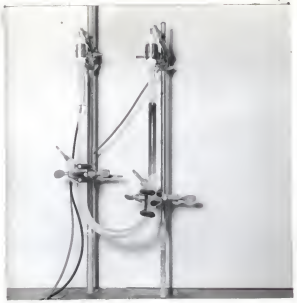

Fig. 6. Appearance of starch-chloral hydrate-furfural coacervated system after electrophoresis for 3 hours at 300 volts. The coacervate was stained with congo red at the end of electrophoresis.

advantage to this method was that the movement of the drops could be observed under the microscope. In addition to the electrophoresis of the drops the disintegration phenomenon and Büchner effect were also observed. The coacervate drops were flattened in a direction perpendicular to the lines of force of the field. The behavior of the coacervate drops was identical to the behavior of the gelatin-gum arabic drops described by Bungenberg de Jong as the Büchner effect (35). He stated that the Büchner effect depended upon the fact that the coacervate had a smaller conductivity than that of the equilibrium liquid.

When the drops were placed in the direct current electrical field, small vacuoles were formed within them. After a short time the small vacuoles united to form large vacuoles, which were transported to the outside of the drop and then their contents were expelled into the equilibrium liquid (Fig. 7). The vacuole contents were completely miscible with the equilibrium liquid and the coacervate drop became rounded off on that side. At the other side of the coacervate drop a turbidity appeared, which upon closer inspection was found to consist of numerous small coacervate droplets (Fig. 7). The formation of large vacuoles continued at a more rapid rate and soon the drop was quite filled with vacuoles. During this time the vacuoles continually broke through to the outside of the drop. The movement of the drop slowed down and became very irregular. This continued until the regular form of the coacervate drop was totally destroyed. All that was left of the large original coacervate drops was a large number of very small coacervate droplets.

It was shown by Bungenberg de Jong and Booij that the disintegration

phenomenon was due to the fact that the two components in the complex coacervate were only loosely bound (9). Thus the two components of the complex coacervate were displaceable in an electrical field. The component with a positive charge moved in the direction of the cathode and the component with a negative charge moved in the direction of the anode. Thus electrophoresis of the two components had taken place within the coacervate drop which led to a separation within the drop.

Bungenberg de Jong assumed that the colloidal ions which were oppositely charged passed through the coacervate surface (10). When a coacervated system, in which the drops were negatively charged was considered, an excess of the negative component was present in the total system. According to analyses made by Bungenberg de Jong this excess was present partly in the equilibrium liquid. The small coacervate droplets were formed in the equilibrium liquid at the cathode side of the drop. At this side of the coacervate drop the positive component which originated from the coacervate was transported into the equilibrium liquid. Thus new coacervate droplets were formed at this site. The negative component which left the opposite side of the drop did not give rise to new coacervate droplets since it was already present in excess in the equilibrium liquid. This was the reason that the small coacervate droplets were formed in the equilibrium liquid exclusively on the cathode side of the original drop.

Separation of the two components was observed within the coacervate drop (Fig. 8). The yellow color of the furfural made possible the detection of this separation. Bungenberg de Jong and de Ruiter considered that local changes of the interfacial tension between coacervate and equilibrium liquid were essential to the explanation of

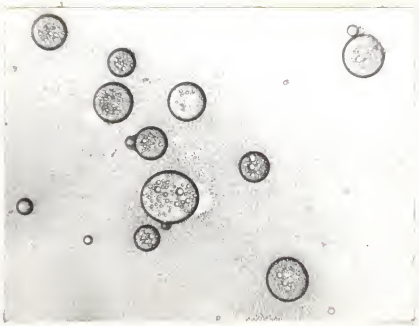

Fig. 7. Appearance of starch-chloral hydrate-furfural coacervate drops showing vacuolation and very small droplets formed as a result of electrophoresis. Mag. 300X

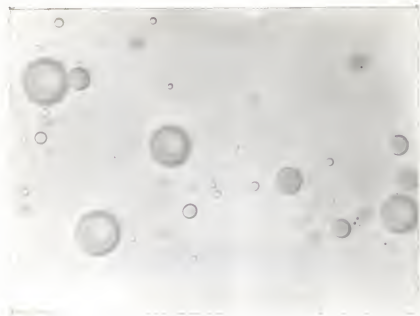

Fig. 8. Starch-chloral hydrate-furfural coacervate drops showing demixing of the components of the coacervate as a result of electrophoresis. Mag. 300X

motory phenomena observed in coacervate drops (17). A micro method for measuring interfacial tension of gum arabic-gelatin coacervate drops was developed by those workers. The interfacial tension was found to depend upon the mixing proportion of the two colloids and at a certain mixing proportion the interfacial tension reached a maximum value. The maximum was obtained at practically the same mixing proportion at which the coacervate volume was at a maximum. Thus at the maximum the difference in composition of the two liquid layers was maximal and this difference became smaller as the limits of coacervation were approached. The hypothesis was advanced that the changes in interfacial tension were due to pH changes caused by polarization of the surface of the drops. Experiments were carried out in order to demonstrate these pH changes in gum arabic-gelatin coacervates (16). No changes in pH were proven, which might have been due to the use of buffered stock sols. These experiments, however, indicated that in the electrical field gum arabic accumulated at the anodal and gelatin at the cathodal side of the drop in consequence of electrophoresis of the components within the drop. Thus the separation of the two components in the drop and not changes in pH were responsible for the changes observed in the interfacial tension.

Voltage settings of 200, 250, 300, 350, 400, 450, and 500 were used to determine the effect of variation of the strength of the electrical field. It was found that the higher the voltage used the more rapidly the drops moved; but the disintegration of the drops also occurred more rapidly than at lower voltage settings. The greater rate of disintegration at the higher field strengths was due to more

rapid electrophoresis of the two components within the drop and to an increase in temperature. It has been shown that as the temperature of the solution containing the coacervate was increased the stability of the coacervate drops was decreased (18). Thus an increase in the temperature of the solution during electrophoresis decreased the stability of the coacervate drops and increased the rate of disintegration.

The ability of a colloid to undergo coacervation has been shown to depend on pH, the mixing proportions of the isohydric sols, the initial concentration of the isohydric sols and the possible presence of indifferent salts (35). It has been shown that the mixing proportion effects the interfacial tension between coacervate and equilibrium liquid. The presence of indifferent salts was under some conditions helpful in coacervate formation and in other cases suppressed coacervation. The pH of the system effects coacervation by determining the extent of ionization of ionizable groups. These factors all have an effect on the charge of the coacervate.

Electrophoresis of Starch-Chloral Hydrate-Gelatin Coacervates

Four-percent of wheat starch (wt./vol.) was dissolved in 6M chloral hydrate solution by heating in a boiling water bath for 45 minutes. The resulting colorless viscous liquid was then mixed with 3% gelatin solution at pH 3.5 in a mixing ratio of 1:1. Coacervation occurred immediately after mixing as a faint cloudiness. The turbidity was due to the formation of coacervate drops which were fluid and of large dimensions.

Two types of electrophoresis were used on this coacervate. One type was free-solution electrophoresis using a U-tube, the second type used a modification of Bungenberg de Jong's apparatus.

When the starch-chloral hydrate-gelatin coacervate was placed in the U-tube, the drops were initially evenly dispersed throughout the solution. After the electrophoresis had proceeded for a period of two hours or longer, depending on the current and voltage setting (Table 3), the drops had formed a layer near the negative electrode. This would indicate that the coacervate drops were positively charged.

The same direction of movement of the drops was observed by use of the modified apparatus of Bungenberg de Jong. The Büchner effect and disintegration phenomenon were observed. The Büchner effect was a flattening of the coacervate drops in a direction perpendicular to the lines of force of the field. It has been shown that the Büchner effect results when the coacervate has a lower conductivity than that of the equilibrium liquid (35).

When the drops were placed in the direct current electrical field, small vacuoles were formed within them. After a short time the small vacuoles united to form large vacuoles, which were transported to the outside of the drop and then their contents were expelled into the equilibrium liquid. The vacuole contents were completely miscible with the equilibrium liquid and the coacervate drop became rounded on that side. At the other side of the coacervate drop a large number of very small coacervate droplets appeared. The formation of the large vacuoles continued at a more rapid rate and soon the drop was quite filled with vacuoles. During this time the vacuoles continually broke through to the outside of the drop. The movement of the drop slowed

down and became very irregular. This continued until the regular form of the coacervate drop was totally destroyed. All that remained of the large original coacervate drop was a large number of very small coacervate droplets. The explanation of the observed phenomena has been discussed in the previous section.

Separation of the two components was observed within the coacervate drop (Fig. 9). It has been shown that this separation of two components is due to electrophoresis which occurs within the drop (16).

Voltage settings of 200, 250, 300, 350, 400, 450, and 500 were used to determine the effect of variation of the field strength. It was found that as the voltage setting was increased the rate at which the drop moved also increased, however the disintegration of the drops also occurred at a faster rate than at a lower voltage setting. The increased rate of disintegration was due to more rapid electrophoresis of the two components within the drop and an increase in temperature. Chung has shown that as the temperature of the solution containing the coacervate was increased the stability of the coacervate drops was decreased (18). Thus an increase in the temperature of the solution during electrophoresis resulted in a decreased stability of the coacervate drops and increased the rate of disintegration.

At pH 3.5 gelatin was well below its isoelectric point. Thus the gelatin was positively charged. The observation that the coacervate of gelatin-starch-chloral hydrate was positively charged was due to the positive charge of the gelatin. Since an opposition of charges is necessary for complex coacervation to take place, the starch-chloral hydrate was negatively charged. Finar pointed out that chloral hydrate in aqueous solution is very acidic and that it has hydrogen bonds

Fig. 9. Starch-chloral hydrate-gelatin coacervate drops showing demixing of the components of the coacervate induced by electrophoresis. Gelatin has accumulated in the dark outer area of the drop and the light inner area of the drop consists of starch-chloral hydrate. Mag. 300X

between two hydroxyl groups and two chloride ions (23). This suggests a possible mode of formation of a "starch-chloral hydrate mixture" as a hydrophilic colloid with an appreciable density of charge and with the consequent ability to combine with positively charged colloidal protein to form coacervate drops. If the production of a coacervate of a hydrophilic colloid depends on the removal of stabilizing factors, capillary charge of the micelle and solvation water surrounding the micelle, then the action of gelatin on the starch-chloral hydrate complex might be explained as a desolvation such as has been studied upon coacervation of gelatin and gum arabic by many workers.

Bungenberg de Jong and Kruyt suggested that the tendency of a hydrophilic colloid to enter into a coacervate was dependent on the density of charge on the surface of the colloid micelle (5, 12). Koets further indicated that the better the opposite charges are balanced and the greater the difference in charge, the greater will be the mutual dehydration (33). Thus a coacervate may separate out in a relatively unstable liquid-rich form or a more stable compact and plastic mass, depending on the charge characteristics of an individual colloid.

SUMMARY AND CONCLUSIONS

A starch coacervate was observed to be formed upon mixing 4% wheat starch (wt./vol.) in 6M chloral hydrate solution and diluting the resulting clear, viscous liquid in a ratio of 1:8 with distilled water at pH 3.5. The coacervate, however, was very unstable and disappeared rapidly into solution within two minutes. The instability of this coacervate was probably due to an excess of negative charge. When the solution was allowed to stand at room temperature after the coacervate had disappeared into solution the formation of a white precipitate was observed. This precipitate could not be regarded as a coacervate since its formation was not influenced by the presence of salts and it showed weak birefringence when viewed on the polarized light microscope. The weak birefringence would indicate that the precipitate had a weak crystalline structure. This precipitate was probably an amylose-chloral hydrate complex as has been suggested by other workers (18, 39).

In an effort to stabilize the unstable coacervate formed at first, 1% solutions of various salts were added before dilution of the starch-chloral hydrate solution with distilled water. When a low concentration of any one of the salts was added, the stability of the resulting coacervate was increased. At higher concentrations of the salt the stability of the coacervate decreased sharply. From the results of this experiment it was concluded that the role of the salt in stabilizing the coacervate was to reduce the negative charge of the starch-chloral hydrate system. Lower concentrations of the divalent and trivalent cations than of the monovalent cations were needed to obtain the same stability. The purpose of this experiment was to prepare a

coacervate with sufficient stability for further studies involving electrophoresis. Since the maximum length of time the coacervate remained stable was too short for any type of electrophoresis to be performed a different method was tried.

When 15% furfural was added to the distilled water before dilution of the 4% starch-chloral hydrate solution (wt./vol.), the resulting coacervate was stable for 24 hours or more. This coacervate was considered to be a complex coacervate involving a microcation and a colloidal anion. It was found that variation of the concentration of the furfural had much the same effect as variation of the concentration of salt added. Since this coacervate was sufficiently stable it was used in the later electrophoresis studies.

Two methods of electrophoresis were used. One method was free solution electrophoresis using a U-tube and the other used a modification of Bungenberg de Jong's apparatus which he used in electrophoresis of gum arabic-gelatin coacervate drops. When the U-tube was used for electrophoresis of the starch-chloral hydrate-furfural coacervate, the coacervate collected in a layer near the positive electrode. When the starch-chloral hydrate-gelatin coacervate was subjected to electrophoresis in the U-tube, the coacervate collected in a layer near the negative electrode. Thus the starch-chloral hydrate-gelatin coacervate was positively charged. The difference in the sign of the charge of the two coacervates was due to the presence of the gelatin in the second coacervate. At pH 3.5 the gelatin was well below its isoelectric point and so had a net positive charge. This would account for the positive charge of the starch-chloral hydrate-gelatin coacervate. The magnitude of the positive charge of the furfural was not as large as that of the

gelatin. Thus the negative charge of the starch-chloral hydrate system was of greater magnitude and the resulting coacervate was negatively charged.

The main advantage of the modified apparatus of Bungenberg de Jong was that the movement of the coacervate drops could be observed under the microscope. The same directions of movement of the coacervate drops were observed using this apparatus. In addition, the Büchner effect and the disintegration phenomenon were observed. The disintegration of the coacervate drops was accounted for by the electrophoresis of the components of the coacervate within the drops. The Büchner effect was explained by the fact that the coacervate had a smaller conductivity than that of the equilibrium liquid. These two phenomona were observed for both the starch-chloral hydrate-furfural coacervate and the starch-chloral hydrate-gelatin coacervate. The starch-chloral hydrate-gelatin coacervate was considered to be a complex coacervate consisting of a colloidal amphoion and a colloidal anion. Thus the formation of both of these coacervates depended on an opposition of charge. Bungenberg de Jong and Kruyt suggested that the tendency of a hydrophilic colloid to enter into a coacervate was dependent upon the density of charge on the surface of the colloidal micelle (5, 12). Thus the better the opposite charges are balanced and the greater the difference in charge, the greater will be the mutual dehydration, and the greater will be the stability of the resulting coacervate. Future work with these coacervates might include the determination of the magnitude of the charge on the drops. This would give information regarding the size of the charge which is optimal

for coacervate formation. Also the role of the chloral hydrate in these coacervates should be investigated to show whether a complex is formed with the starch or a chemical reaction occurs.

ACKNOWLEDGMENTS

The writer wishes to express his appreciation to Dr. Majel M. MacMasters, for directing this investigation and for advice on the preparation of manuscripts; and to Professor Gerald D. Miller of this department and to Dr. R. Kenneth Burkhard of the Department of Biochemistry for their advice and encouragement.

The writer is also indebted to the Corn Industries Research Foundation for the generous support which made this investigation possible and to Dr. William J. Hoover, Head of the Department of Grain Science and Industry, for his interest and support of this work. Thanks also to my wife, Jackie, for her invaluable help in the preparation and typing of this manuscript.

REFERENCES

1. Bamford, C. H., and Tompa H.
The theory of coacervation. Trans. Faraday Soc. 46: 310-316 (1950).

2. Basu, Sadhan, and Bhattacharya, Gurucharan
Some aspects of the phenomenon of coacervation. Science 115: 544-545 (1952).

3. Bice, C., MacMasters, M. M., and Hilbert, G. E.
Proposed use of starch sponges as internal surgical dressings absorbable by the body. Science 100: 227 (1944).

4. Böeseken, J.
Some remarks on the condensation products of polyoxy-compounds with chloral. Koninkl. Akad. Wetenschap. Amsterdam 30: 55-60 (1927).

5. Bungenberg de Jong, H. G.
Coacervation and its importance in biology. Expos. Biol. (Paris) 397: 1-99 (1936).

6. Bungenberg de Jong, H. G.
Distribution of the complex component which is present in excess, between complex coacervate and equilibrium liquid. Koninkl. Akad. Wetenschap. Amsterdam 50: 707-711 (1947).

7. Bungenberg de Jong, H. G.
Behavior of microscopic bodies consisting of biocolloid systems and suspended in an aqueous medium. VI. A. Auxiliary apparatus for studying the morphological changes of coacervate drops. B. Preparation and behavior of composite drops consisting of coexisting complex coacervates. Koninkl. Akad. Wetenschap. Amsterdam 45: 393-400 (1942).

8. Bungenberg de Jong, H. G.
Oriented coacervates and their bearing upon the formation of colloid-crystals. Koninkl. Akad. Wetenschap. Amsterdam 38: 426-434 (1935).

9. Bungenberg de Jong, H. G. and Booij, H. L.
Biocolloids and their interactions. Protoplasmatologia IV: 1-162 (1956).

10. Bungenberg de Jong, H. G., and Hoskam, E. G.
Motory phenomena in coacervate drops in a diffusion field and in the electric field. Koninkl. Akad. Wetenschap. Amsterdam 44: 1099-1111 (1941).

11. Bungenberg de Jong, H. G., and Hoskam, E. G.
Effect of neutral salts on the composition of complex coacervate (gelatine + gum arabic) and equilibrium liquid at constant pH and constant mixing proportion of the two colloids in the total system. Koninkl. Akad. Wetenschap. Amsterdam 45: 59-66 (1942).

12. Bungenberg de Jong, H. G., and Kruyt, H. R.
Extension of the theory of complex coacervation to ionical disperse systems. Koninkl. Akad. Wetenschap. Amsterdam 38: 714-722 (1935).

13. Bungenberg de Jong, H. G., and Landsmeer, J. M. F.
Diameter changes of gelatinized coacervate drops of the complex coacervate gelatine-gum arabic, resulting from a change in the pH of, or the addition of neutral salts to the surrounding medium. Rec. trav. chim. 65: 606-613 (1946).

14. Bungenberg de Jong, H. G., and Linde, P. V. D.
Coacervate sols and their relation to the theory of lyophilic colloidal stability. Koninkl. Akad. Wetenschap. Amsterdam 38: 419-426 (1935).

15. Bungenberg de Jong, H. G., and Recourt, A.
Binding of the oppositely charged salt ions to the soap ions in the formation of elastic systems and in coacervates I. Koninkl. Akad. Wetenschap. Amsterdam 56: 303-314 (1953).

16. Bungenberg de Jong, H. G., and de Ruiter, L.
Contribution to the explanation of motory and disintegration phenomena in complex coacervate drops in the electric field. Koninkl. Akad. Wetenschap. Amsterdam 50: 1189-1200 (1947).

17. Bungenberg de Jong, H. G., and de Ruiter, L.
The interfacial tension of gum arabic-gelatin complex--coacervates and their equilibrium liquids. Koninkl. Akad. Wetenschap. Amsterdam 50: 836-848 (1947).

18. Chung, Hsin Yau, and MacMasters, M. M.
Coacervation of starch III. Complex coacervation of starch with gelatin. Die Stärke 18: 377-382 (1966).

19. Dedek, J., Jelinek, B., and Kulcickyj, I.
Studies on the gelatinization of starch. Ann. Fermentations II, 400 (1936).

20. Dobry, A.
La Coacervation. Rev. Can. Biol. 1: 353-365 (1942).
English translation by MacMasters, M. M.

21. Dobry, A.
Recherches sur la coacervation V. Fractionnement des macromolecules par coacervation. J. Chim. Phys. 42 (10): 109-113 (1945). English Translation by MacMasters, M. M.

22. Evreinova, T. N., Shurygina, N. N., and Oparin, A. I.
Starch synthesis in coacervate drops. Biokhimiya 29 no. 6: 1035-1041 (1964).

23. Finar, I. L.
Organic Chemistry. Vol. 1, London: Longmans (1959). p. 157.

24. Fouard, E.
Concerning the properties of starch in relationship to its colloidal form. Compt. rend. 146: 978-981 (1908).

25. Fouard, E.
On the colloidal properties of starch and on the existence of a perfect solution of that substance. Compt. rend. 146: 285-287 (1908).

26. Fouard, E.
On the colloidal properties of starch and on the unity of its constitution. Compt. rend. 147: 813-816 (1908).

27. Gavoret, G., and Duclaux, J.
Macromolécules et coacervation. J. Chim. Phys. 41: 45-48 (1944). English translation by MacMasters, M. M.

28. Gavoret, G., and Duclaux, J.
Macromolécules et coacervation. III. Fractionnement. J. Chim. Phys. 42: 44-51 (1945). English translation by MacMasters, M. M.

29. Gilbert, G. A.
Sedimentation and electrophoresis of interacting substances I. Idealized boundary shape for a single substance aggregating reversibly. Proc. Roy. Soc. (London) 250A: 377-388 (1959).

30. Guiliano, Raffaele, and Stein, M. L.
Identification of some starches by differential staining. Ann. Chim. (Rome) 45: 236-240 (1955).

31. Guilliermond, A.
On a method for staining starch granules within the mitochondria in plant cells. Compt. rend. soc. biol. (Paris) 79: 806-809 (1916).

32. Koets, P.
Complex coacervation of amylophosphoric acid and proteins and its possible bearing on the problem of amylopectin. Koninkl. Akad. Wetenschap. Amsterdam 38: 63-74 (1935).

33. Koets, P.
Coacervation of amylophosphoric acid and proteins. J. Phys. Chem. 40: 1191 (1936).

34. Kossel, A.
The protamines and histones. Monograph, Longmans Green and Co. (1928).

35. Kruyt, H. R.
Colloid Science II. Elsevier Publishing Co., New York (1949).

36. Langmuir, Irving
The role of attractive and repulsive forces in the formation of tactoids, thixotropic gels, protein crystals and coacervates. J. Chem. Phys. 6: 873-896 (1938).

37. Lepeschkin, W.
Study of the chemical reactions during the swelling of starch in hot water. Bull. Soc. Botan. Géneve 13: 40-65 (1921).

38. MacMasters, M. M., Eck, J. W., and Hilbert, G. E.
Some factors affecting starch paste viscosity. Manuscript presented at the Annual Meeting of A.A.C.C. in Kansas City, Mo., May 19-23 (1947).

39. MacMasters, M. M., Cox, M. J., Eck, J. W., and Bice, C. W.
Coacervation of starch. Manuscript presented at the Annual Meeting of A.A.C.C. in Niagara Falls, Ontario, Canada, May 13-16 (1946).

40. Maige, A.
Influence de la temperature sur la formation de l'amidon dans les cellules vegetales. Compt. rend. soc. biol. 86: 685-686 (1922). English translation by MacMasters, M. M.

41. Maige, A.
Remarques au sujet de la persistance de l'écorce plastidale autour des grains d'amidon. Compt. rend. soc. biol. 95: 299-301 (1926). English translation by MacMasters, M. M.

42. Maige, A.
Variations du seuil de condensation amylogene avec la temperature. Compt. rend. soc. biol. 90: 685-687 (1924). English translation by MacMasters, M. M.

43. Maquenne, L., and Roux, Eug.
Sur la constitution, la saccharification et la retrogradation des empois de fécule. Compt. rend. 140: 1303-1308 (1905). English translation by MacMasters, M. M.

44. Maquenne, L., Fernbach, A., and Wolff, J.
Retrogradation and coagulation of starch. Compt. rend. 138: 49-51 (1904). English translation by MacMasters, M. M.

45. McBain, J. W.
Colloid Science. D. C. Heath and Co., Boston (1950).

46. McGraw-Hill
Coacervation. Encyclopedia of Science and Technology 3: 227-228 (1960).

47. Michaeli, I., Overbeek, J. Th. G., and Voorn, M. J.
Phase separation of polyelectrolyte solutions. J. Polymer Sci. 23: 443-450 (1957).

48. Petit, P. and Richard
On the mechanical liquefaction of starch paste. Compt. rend. 182: 657-659 (1926).

49. Ostwald, Wo., and Hertel, R. H.
Kolloidchemische Reaktionen zwischen Solen von Eiweisskörpern und polymeren Kohlehydraten. I. Kolloid Z. 47: 258-268 (1929).

50. Ostwald, Wo., and Hertel, R. H.
Kolloidchemische Reaktionen zwischen Solen von Eiweisskörpern und polymeren Kohlehydraten. II. Kolloid Z. 47: 357-370 (1929).

51. Reed, Howard S., and Dufrenoy, Jean
Catechol aggregates in the vacuoles of cells of zinc deficient plants. Am. J. Botany 29 no. 7: 544-551 (1942).

52. Reed, H. S., and Dufrenoy, J.
Coacervates in physical and biological systems. Phytopathology 32 no. 7: 568-579 (1942).

53. Reed, H. S., and Dufrenoy, J.
Coacervates in physical and biological systems. A. The production of coacervates. Phytopathology 33 no. 8: 739-741 (1943).

54. Rendleman, Jacob A.
Alkali metal complexes of carbohydrates I. Interaction of alkali metal salts with carbohydrates in alcoholic media. J. Org. Chem. 31: 1839-1845 (1966).

55. Rendleman, Jacob A.
Alkali metal complexes of carbohydrates II. Interaction of bases with carbohydrates in alcoholic media. J. Org. Chem. 31: 1845-1851 (1966).

56. Rosenfeld, Samuel S.
Starch sponge--a hemostatic agent. Bull. N. Y. Acad. Med. 25 no. 7: 453-454 (1949).

57. Roux, Eug.
Sur la rétrogradation et la composition des amidons naturels autres que la fécule. Compt. rend. 142: 95-97 (1906). English translation by MacMasters, M. M.

58. Sutra, R.
Gelatinization, grinding and homogenity of starch. Bull. soc. chim. 738-741 (1947).

59. Voorn, M. J.
Complex coacervation I. General theoretical considerations. Rec. trav. chim. 75: 317-330 (1956).

60. Wolff, J., and Fernbach, A.
On the coagulation of starch. Compt. rend. 137: 718-719 (1903).

61. Wolff, J., and Fernbach, A.
Researches on the coagulation of starch. Ann. inst. Pasteur 18: 165-180 (1904). English translation by MacMasters, M. M.

62. Wolff, J., and Fernbach, A.
De l'influence de quelques composés minéraux sur la liquéfaction des empois de fécule. Compt. rend. 143: 363-365 (1906). English translation by MacMasters, M. M.

63. Yu, David T., and MacMasters, M. M.
Coacervation of starch II. Some instances. Die Stärke 17: 75-77 (1965).

COACERVATION OF STARCH

by

ROBERT EARL BAKER

B. S., Kansas State University, 1965

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Grain
Science and Industry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1967

ABSTRACT

Coacervation is a colloidal phenomenon in which a phase separation occurs. The phase which is rich in colloidal material is the coacervate. The phase which is poor in colloidal material is the equilibrium liquid. A coacervate results from an action which removes the stabilizing factors of a hydrophilic colloid (e.g. by a change in pH, addition of neutral salt, addition of a colloid of opposite charge, etc.). A coacervate may be obtained as spherical drops or as large sheets which may be visible under the microscope.

Several instances of starch coacervation have been demonstrated, though there is an adverse suggestion that starch fails to form a coacervate. Starch sponge which is produced by coacervation of starch paste by freezing is one of the most practically successful applications of starch coacervates.

This investigation presents the results of further work on the conditions leading to the formation of starch coacervates and on the properties of these coacervates. Solutions of 4% starch (wt./vol.) dissolved in 6M chloral hydrate when diluted with distilled water gave only extremely unstable coacervate drops which upon standing rapidly disappeared into solution. A few hours later a white precipitate formed which was believed to be an amylose-chloral hydrate complex.

In an effort to stabilize the extremely unstable coacervate formed at first, various concentrations of different salts were added singly before dilution of the starch-chloral hydrate solution. Low concentration of any of the salts increased the stability of the resulting coacervate. At higher concentrations of salt, the coacervate was more unstable

than when no salt was added.

When 15% furfural was added to the distilled water before the starch-chloral hydrate solution was diluted, the resulting coacervate was stable for 24 hours or longer. Since the length of time the coacervate remained stable was sufficiently long, electrophoresis studies were performed with this coacervate.

The results of electrophoresis of starch-chloral hydrate-furfural coacervates and starch-chloral hydrate-gelatin coacervates were presented. The starch-chloral hydrate-furfural coacervate was found to have a net negative charge. The starch-chloral hydrate-gelatin coacervate was found to have a net positive charge. The positive charge of the starch-chloral hydrate-gelatin coacervate was due to the strong positive charge of the gelatin at the pH used. Disintegration phenomonon and the Büchner effect were observed with both of these coacervates.

From the results of electrophoresis the starch-chloral hydrate-furfural coacervate was postulated to be a complex coacervate involving a microcation and a colloidal anion. The starch-chloral hydrate-gelatin coacervate was postulated to be a complex coacervate involving a colloidal cation and a colloidal anion. A complex coacervate results from the mutual dehydration of the two components of the coacervate due to the opposition of charges. Thus the greater the difference in charge and the better the opposite charges are balanced, the greater will be the mutual dehydration and the greater will be the stability of the resulting coacervate. Therefore the tendency of a hydrophilic colloid to enter into a coacervate is dependent upon the density of charge on the surface of the colloid micelle.